and Hornsby, 1999, Biotechnol. Prog. 15:763-767) Once the microspheres are formed using double-emulsification/solvent evaporation (Alonso, et al., 1993, Pharmacol. Res. 10:945-953), the carbobenzoxy (i.e., CBZ) protective groups are removed using either acid hydrolysis or lithium/liquid ammonia reduction, thereby exposing reactive amine groups. Lithium/liquid ammonia reduction is recommended if microspheres are desired, given its less harsh effect of the external surface of the microparticle. In addition, the lithium treatment was reported to be more effective in producing surface reactive amino groups than was the acid hydrolysis procedure. If a solid surface particle (i.e., a microsphere) is desired, the lithium treatment may be preferred. In this latter method, the active agent may be added during the formation of the microparticles since the lithium treatment reportedly does not create pores in the surface of the particles and thus will not adversely affect the agent. If, on the other hand, a surface porous particle is desired, then the acid hydrolysis method may be preferred, provided the agent is either resistant to the acid treatment or is loaded into the particles following acid treatment.

B2 Cont

In the Claims

Please re-write the claims as indicated below. A marked-up copy of the amended claims is attached hereto as Appendix A.

B3

1. (Amended) A method of treating a subject to attach microparticles to a skin surface containing endogenous transglutaminase of the subject comprising

contacting the skin surface containing endogenous transglutaminase with microparticles having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the microparticles to the skin surface in the presence of the endogenous transglutaminase,

allowing the microparticles to remain in contact with the skin surface for a time sufficient to permit a layer of microparticles to covalently attach to the skin surface.

2. The method of claim 1, wherein the surface available transglutaminase substrate reactive groups are lysines.

3. The method of claim 1, wherein the surface available transglutaminase substrate reactive groups are glutamines.

Pls/rtn

4. The method of claim 1, wherein the layer of microparticles is non-planar.

5. The method of claim 1, wherein the microparticles further comprise an active agent.

6. The method of claim 5, wherein the active agent is a non-nucleic acid active agent.

7. The method of claim 5, wherein the active agent is a non-protein active agent.

8. The method of claim 5, wherein the active agent is selected from the group consisting of a cosmetic agent, a bulking agent, a hair conditioning agent, a hair fixative, a sunscreen agent, a moisturizing agent, a depilatory agent, an anti-nerve gas agent, a film forming agent, a vitamin, an insect repellant, a coloring agent, a pharmaceutical agent, a ligand-receptor complex and a receptor of a ligand-receptor complex.

9. The method of claim 5, wherein the active agent is not itself a substrate of transglutaminase.

10. The method of claim 1, wherein the microparticles further comprise a synthetic polymer.

11. The method of claim 10, wherein the synthetic polymer is latex.

12. The method of claim 10, wherein the synthetic polymer is polystyrene.

13. The method of claim 1, wherein the microparticles are porous.

14. The method of claim 1, wherein the microparticles are 100 nm to 500 nm in size.

Duplicate

15. The method of claim 1, wherein the microparticles are 20 nm to 35 nm in size.

16. The method of claim 1, wherein the microparticles are non-biodegradable.

17. The method of claim 1, wherein the microparticles are detergent insoluble.

18. The method of claim 1, wherein the transglutaminase substrate reactive groups are part of a polymer.

19. The method of claim 18, wherein the polymer is covalently attached to the microparticle.

20. The method of claim 18, wherein the polymer is comprised of at least 50% lysines.

21. The method of claim 18, wherein the polymer is lysine-rich at a surface available terminus.

22. (Amended) The method of claim 18, wherein the polymer comprises a polymer selected from the group consisting of polymers containing:

(a) at least two contiguous linked lysines,

(b) at least three contiguous linked lysines,

(c) at least four contiguous linked lysines, and

(d) at least five contiguous linked lysines.

23. The method of claim 18, wherein the polymer is comprised of at least 50% glutamines.

24. The method of claim 18, wherein the polymer is glutamine-rich at a surface available terminus.

25. (Amended) The method of claim 18, wherein the polymer comprises a polymer selected from the group consisting of polymers containing:

(a) at least five contiguous linked glutamines,

(b) at least ten contiguous linked glutamines,

(c) at least fifteen contiguous linked glutamines, and

(d) at least twenty contiguous linked glutamines.

26. (Amended) A method of treating a subject to attach nonlabeling microparticles to a skin surface of the subject comprising

contacting the skin surface with nonlabeling microparticles having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the nonlabeling microparticles to the skin surface in the presence of exogenous transglutaminase,

applying exogenous transglutaminase to the skin surface, and

allowing the nonlabeling microparticles and exogenous transglutaminase to remain in contact with the skin surface for a time sufficient to permit a layer of nonlabeling microparticles to covalently attach to the skin surface.

51. A kit comprising

a microparticle comprising surface available transglutaminase substrate reactive groups in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase, and

instructions for topically administering the microparticle to a skin surface.

75. The kit of claim 51, wherein the microparticle is provided in a topically administered form selected from the group consisting of an ointment, an aerosol, a gel, and a lotion.

76. (Amended) A kit comprising

a nonlabeling microparticle having surface available transglutaminase substrate reactive groups in an amount sufficient to attach the nonlabeling microparticle to a skin surface in the presence of exogenous transglutaminase, and

instructions for topically administering the nonlabeling microparticle and transglutaminase to a skin surface.

77. The kit of claim 76, wherein the kit further comprises transglutaminase.

102. A composition comprising

a microparticle comprising an active agent and a lysine-rich polymer having transglutaminase substrate reactive groups, wherein the microparticle is non-biodegradable, and the transglutaminase substrate reactive groups are surface available.

117. The composition of claim 102, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of endogenous transglutaminase.

118. The composition of claim 102, wherein the transglutaminase substrate reactive groups are surface available in an amount sufficient to attach the microparticle to a skin surface in the presence of exogenous transglutaminase.

119. The composition of claim 102, wherein the lysine-rich polymer comprises a polymer of amino acids and wherein at least 50% of the amino acids are lysine.

123. A composition comprising